When deacetylation was allowed to occur at the same *p*H but at 5.0 ± 0.2° and the spectrum was scanned from 270–230 mμ both before and, at a series of times, after the addition of base, the resulting difference spectra (inset to Fig. 2) show the rapid appearance of a peak with its maximum at 245 mμ which slowly declines with time, corresponding closely to that described for acetyl-imidazole.[4] According to the published extinction coefficient of this compound[4] ($\epsilon = 3 \times 10^3$), the observed maximum increase and subsequent decrease at 245 mμ is equivalent to 0.41–0.42 mole acetylimidazole per mole of reactive acetyl in the enzyme. Similar results were obtained in glycine buffer, but in phosphate the magnitude of the change at 245 mμ was reduced.

It is postulated, therefore, that as indicated in Fig. 1, the deacetylation of mono-acetyl-δ-chymotrypsin occurs by a rapid intramolecular transfer of acetyl- from serine hydroxyl to imidazolyl- followed by a slower hydrolysis of acetyl-imidazolyl-. The first order rate constant for the disappearance of the E_{245} compound corresponds closely with that observed for the rate of deacetylation of δ-chymotrypsin as measured by the reappearance of enzyme activity,[6] which in turn corresponds to the rate of base catalyzed hydrolysis of acetyl-imidazole in a model system.[8]

(8) M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1656 (1957).

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SYNTHESIS OF POTENTIAL ANTICANCER AGENTS. X. 2-FLUOROADENOSINE[1]

Sir:

Recently the biological activity of three fluoro derivatives of naturally occurring pyrimidines has been reported.[2]

Of these three fluoropyrimidines, 5-fluorouracil and 5-fluoroörotic acid have shown appreciable tumor-inhibitory activity against a variety of rat and mouse tumors[2a] and 5-fluorouracil was selected for clinical trials.[2b] The biological activity of the fluoropyrimidines increased our interest in the preparation of fluoropurines and their ribosides, especially fluoro derivatives of naturally occurring purines. Although Bendich, Giner-Sorolla and Fox were unable to prepare 6-fluoropurine from adenine by the Schiemann reaction,[3] Weisbach successfully prepared 2-fluoropyrimidine from 2-aminopyrimidine by this method.[4] The success of the latter reaction led us to attempt the preparation of 2-fluoroadenosine from 2,6-diaminopurine riboside by diazotization in fluoboric acid.

An aqueous solution of sodium nitrite (360 mg. in 2.4 ml.) was added with stirring to a solution of 2,6-diaminopurine riboside[5] (846 mg.) in 48% fluoboric acid (9.6 ml.) at −10°. The solution was stirred at −10° to 0° for 15 minutes, cooled to −20° and neutralized with 50% sodium hydroxide solution. The water was removed *in vacuo* and the residue chromatographed on a Celite column using water-saturated butanol. The crude 2-fluoroadenosine obtained (149 mg.) was recrystallized from absolute ethanol and dried *in vacuo* over P_2O_5 at 70° for several hours: yield, 75 mg. (8.7%), dec. at 200°; $(\alpha)^{26}D$ −60.3 ± 11.1 (0.127% in ethanol); $\lambda_{max}^{pH\ 1}$ 260.5 mμ (a_M 13,700); $\lambda_{max}^{pH\ 7}$ 260.5 mμ (a_M 14,300); $\lambda_{max}^{pH\ 13}$ 260.5 (a_M 14,800). *Anal.* Calcd. for $C_{10}H_{12}FN_5O_4 \cdot {}^1/_4C_2H_5OH$: C, 42.45; H, 4.60; N, 23.60. Found: C, 42.34; H, 4.93; N, 23.40. A qualitative test for fluorine was positive. The ratio of the R_f values of 2-fluoroadenosine and adenine in butanol–water on a descending paper chromatogram (Watman No. 1) was 0.9.

2-Fluoropurine was prepared in the same manner from 2-aminopurine[6] (850 mg.): yield, 254 mg. (41%) dec. at 216°; $\lambda_{max}^{pH\ 1}$ 264 mμ (a_M 8,300) $\lambda_{max}^{pH\ 7}$ 266.5 mμ (a_M 8,400), $\lambda_{max}^{pH\ 13}$ 272 mμ (a_M 8,800). *Anal.* Calcd. for $C_5H_3FN_4$: C, 43.48; H, 2.20; N, 40.60. Found: C, 43.52; H, 2.01; N, 40.37. A qualitative test for fluorine was positive.

In preliminary tests 2-fluoroadenosine inhibits the growth of Human Epidermoid Carcinoma (HE 2) at 10^{-8} g./ml. Five times this concentration is required to inhibit monkey kidney cells. Azaserine and 6-diazo-5-oxo-L-norleucine inhibit the growth of these tissues at 10^{-7} g./ml.

The preparation of other 2-fluoropurines is now under way in this laboratory.

(5) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 1650 (1951).
(6) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).
(7) Affiliated with Sloan-Kettering Institute.

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THE SYNTHESIS OF 5-FLUOROPYRIMIDINES

Sir:

We wish to report the synthesis of a new class of compounds, some of which were designed to function as nucleic acid antagonists, by substituting fluorine for hydrogen in naturally occurring pyrimidines.

The 5-fluoropyrimidines (III) were obtained from pseudourea and pseudothiourea salts (I) and α-fluoro-β-keto ester enolates (II) by adaptation of the Wheeler synthesis.[1]

Crystalline IIa was prepared by the addition at 0° of 2.4 moles of methyl formate and 1.2 moles of ethyl fluoroacetate (IV) to 1.2 moles of potassium ethoxide in 800 ml. of toluene and letting the mix-

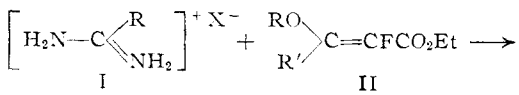
(1) H. L. Wheeler and H. F. Merriam, *Am. Chem. J.*, **29**, 478 (1903); A. Dornow, F. Boberg and L. Schürer, *Arch. Pharm.*, **286**, 494 (1953).

(1) This work was supported by funds from the C. F. Kettering Foundation. Part IX, John A. Montgomery and Carroll Temple, Jr., THIS JOURNAL, in press.

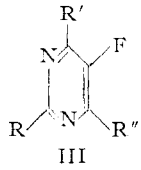
(2) (a) C. Heidelberger, D. Morren, L. Griesbach, B. J. Montag, R. Duschinsky, E. Pleven and R. Schnitzer, *Proc. Am. Ass. Cancer Research*, **2**, 212 (1957); (b) F. A. McIver, A. R. Curreri, O. O. Meyer, R. F. Schilling and H. Waisman, *ibid.*, **2**, 230 (1957); (c) C. Heidelberger, L. Bosch, N. K. Chaudhuri and P. B. Danneberg, *Federation Proc.*, **16**, 194 (1957); (d) J. M. Scheiner, E. Kostelak and R. Duschinsky, *ibid.*, **16**, 242 (1957); (e) T. Wong and W. M. Benson, *ibid.*, **16**, 348 (1957).

(3) A. Bendich, A. Giner-Sorolla and J. J. Fox, "The Chemistry and Biology of Purines" (A Ciba Foundation Symposium), J. and A. Churchill Ltd., London, England, 1957, p. 7.

(4) D. E. Weisbach, M. S. Thesis, University of North Carolina, 1954.



I II



III

I	a	b	c	II	a	b	c	d
R =	SEt	OMe	SMe	R =	K	Na	K	H
X =	Br	Cl	$^1/_2SO_4$	R′ =	H	H	CO_2Et	CH_2F

III	a	b	c	d	e	f	g	h	i	j	k	l
R =	SEt	OH	OMe	SMe	SH	SEt	SEt	OH	SEt	OH	SMe	OH
R′ =	OH	OH	OH	OH	OH	Cl	NH_2	NH_2	OH	OH	OH	OH
R″ =	H	H	H	H	H	H	H	H	CO_2Et	CO_2H	CH_2F	CH_2Cl

ture stand at 25° for 16 hours.[2] After IIa was refluxed for 2 hours with 0.6 moles of Ia and 0.6 moles of sodium methoxide in 1440 ml. of ethanol, evaporation, extraction with water and acidification gave IIIa (24% yield from IV), m.p. 192–193° dec. (recrystallized from ethyl acetate) (Calcd. for $C_6H_7FN_2OS$: C, 41.38; H, 4.05; F, 10.91. Found: C, 41.45; H, 4.25; F, 11.48.) Hydrochloric acid hydrolysis[1] of IIIa afforded 72% of 5-fluorouracil (IIIb), m.p. 282–283° dec., $\lambda_{max}^{0.1N\ HCl}$ 265–266 mμ (ε 7070)[3] (Calcd. for $C_4H_3FN_2O_2$: C, 36.93; H, 2.32; F, 14.61. Found: C, 37.07; H, 2.30; F, 14.69). Similarly Ib and IIa gave IIIc, m.p. 206–207° dec. (Calcd. for $C_5H_5FN_2O_2$: C, 41.67; H, 3.50; OCH_3, 21.63; F, 13.18. Found: C, 42.01; H, 3.87; OCH_3, 21.70; F, 13.51). This was hydrolyzed to yield IIIb. Hydrogenation of IIIb (1 mole hydrogen) with palladium charcoal in 2 moles of sodium hydroxide yielded 80% of uracil, whereas rhodium catalyst[4] in acetic acid produced a mixture from which 6.5% of 5-fluorodihydrouracil, m.p. 237–238° dec. (Calcd. for $C_4H_5FN_2O_2$: C, 36.37; H, 3.82; F, 14.38. Found: C, 36.32; H, 3.43; F, 14.59) was isolated by cellulose powder chromatography.[5] Condensation of Ic and IIa gave IIId, m.p. 241–243° dec. (Calcd. for $C_5H_5FN_2OS$: C, 37.49; H, 3.15; N, 17.49. Found: C, 37.98; H, 3.44; N, 17.52) which on demethylation[6] afforded 49% of IIIe, m.p. 227–229° dec. (Calcd. for $C_4H_3FN_2OS$: C, 32.87; H, 2.07; F, 13.00; Found: C, 33.45; H, 2.36; F, 12.78). Chlorination[7] of IIIa produced oily IIIf, which by autoclaving (12 hours, 100°) with liquid ammonia gave IIIg, m.p. 94°, in 88% over-all yield (Calcd. for $C_6H_8FSN_3$: C, 41.60; H, 4.66; N, 24.26. Found: C, 41.43; H, 4.73; N, 23.96). Hydrobromic acid hydrolysis of IIIg afforded 52% of 5-fluorocytosine (IIIh), m.p. 295–297° dec., $\lambda_{max}^{0.1N\ HCl}$ 285 mμ (ε 8900) (Calcd. for $C_4H_4FN_3O$: C, 37.21; H, 3.12; F, 14.72. Found: C, 36.92; H, 3.07; F, 14.47).

Diethyl oxalate (2 moles), potassium ethoxide and IV gave IIc[8] (Calcd. for $C_8H_{10}FKO_5$: C, 39.34; H, 4.12; K, 16.01; F, 7.78. Found: C, 39.03; H, 4.34; K, 16.48; F, 7.59). Condensation (as described for IIa) of Ia and IIc yielded, after processing IIIi (23% from IV), m.p. 168–169° dec. (Calcd. for $C_9H_{11}FN_2O_3S$: C, 43.89; H, 4.50. Found: C, 43.96; H, 4.61). Hydrochloric acid hydrolysis of IIIi yielded 88% of 5-fluoroörotic acid monohydrate (IIIj) m.p. 255° dec., $\lambda_{max}^{0.1N\ HCl}$ 284–285 mμ (ε 7100)[3] (Calcd. for $C_5H_3FN_2O_4,H_2O$: C, 31.26; H, 3.13; F, 9.89. Found: C, 31.36; H, 2.95; F, 10.11), which on refluxing in Dowtherm yielded 86% of IIIb.[9] Condensation of Ic and IId[10] (2 moles sodium methoxide) gave IIIk m.p. 221–222° dec. which was impure, due to partial loss of side chain fluorine (Calcd. for $C_6H_6F_2N_2OS$: C, 37.49; H, 3.15; F, 19.77. Found C, 37.68; H, 2.79; F, 13.01). This upon refluxing with hydrochloric acid yielded III (40% over-all yield from IId) m.p. 240–241° dec. (Calcd. for $C_5H_4ClFN_2O_2$: C, 33.63; H, 2.26; Cl, 19.86; F, 10.64. Found: 34.03; H, 2.11; Cl, 19.47; F, 10.64).

5-Fluorouracil and 5-fluoroörotic acid have profound activity[11] against bacteria *in vitro* and against several transplanted tumors in animals. The former is under clinical investigation in neoplastic diseases.

We are indebted to Mrs. Ellen Chiamulera for technical assistance and to Dr. Al Steyermark for the microanalyses.

(2) The compound is impure and unstable; it should be used without undue delay in the next step. In a similar run with sodium ethoxide the obtained IIb was converted by treatment with ethanolic hydrochloric acid at 25° into ethyl fluoromalonaldehydate diethyl acetal (9.3% from IV), b.p. 115–118° (24 mm.), $n^{25}D$ 1.4041. (Calcd. for $C_9H_{17}FO_4$: C, 51.91; H, 8.23; F, 9.12; OC_2H_5, 64.92. Found: C, 52.19; H, 8.38; F, 9.02; OC_2H_5, 64.55.)

(3) Data supplied by Dr. A. Motchane.

(4) W. E. Cohn and D. G. Doherty, THIS JOURNAL, **78**, 2863 (1956).

(5) The upper phase of a mixture of ethyl acetate, water, formic acid (60:35:5) was used as eluant. *Cf.* K. Fink, R. E. Cline, R. B. Henderson and R. M. Fink, *J. Biol. Chem.*, **221**, 430 (1956). The collaboration of Mr. W. E. Oberhansli in the chromatographic work is gratefully acknowledged.

(6) H. W. Barrett, I. Goodman and K. Dittmer, THIS JOURNAL, **70**, 1755 (1948).

(7) H. L. Wheeler and T. B. Johnson, *Am. Chem. J.*, **29**, 496 (1903).

(8) *Cf.* I. Blank, J. Mager and E. D. Bergmann, *J. Chem. Soc.*, 2192 (1955).

(9) This method produced 2-C^{14} labeled IIIb from Ia via IIIj.

(10) E. T. McBee, O. R. Pierce, H. W. Kilbourne and E. R. Wilson, THIS JOURNAL, **75**, 3152 (1953).

(11) C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven and J. Scheiner, *Nature*, **179**, 663 (1957).

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RECEIVED JULY 1, 1957

SOME SELECTIVE REACTIONS OF THE SILICON-HYDROGEN GROUP WITH ORGANOMETALLIC COMPOUNDS

Sir:

We are reporting a series of reactions which readily make available the synthesis of a wide variety of organosilicon compounds, particularly those of an unsymmetrical nature. The introduction of the various R groups can be effected stepwise by the proper choice of solvent and organometallic compound. The synthesis is particularly appropriate for the preparation of low-melting organosilicon compounds of the type R_4Si where all of the R groups can be different.

Previous reports have shown that organolith-